Introduction

Microarray analysis is becoming an increasingly popular tool for examining the changing expression profiles of thousands of genes after exposure to toxicants. However, since the results may vary according to the method employed, one needs to be careful of the final interpretation of the data. One area where microarray technology has the potential for generating useful data is in sulfur mustard vesicant research. In this field microarray technology might be used to identify potential new target molecules or pathways for medical intervention with potential countermeasures to injury. Another use could be the identification of biomarkers that could be used as quantitative tools to quickly evaluate the success of novel compounds to alleviate vesicle injury or enhance wound repair. Sulfur mustard [bis[chloroethyl]sulfide (sulfur mustard, SM)] is a potent alkylating agent which penetrates the skin rapidly and causes skin blistering within hours. This blistering is at the level of the dermo-epidermal junction, which, interestingly enough is the identical pathological target of junc-tional epidermolysis bullosa (JEB), a genetic skin blistering disease where the epidermis separates away from the dermis, thus compromising the skin integrity. Disruption of the dermo-epidermal junction in JEB will be further enhanced by the actions of matrix metalloproteinases (MMPs), a family of enzymes known both to enhance the action of many activating factors during the inflammatory response, as well as cause further degradation to the tissue. Our previous work identified matrix metalloproteinase-9 (MMP-9) as one in particular that quantitatively increases over time in response to sulfur mustard exposure (Shakarjian J Appl Tox 26(3):239-246, 2006). Since this increase in MMP-9 corresponded to increased tissue damage, we hypothesized that a quantitative reduction of it in skin would reduce the tissue damage normally observed after SM exposure. We tested two separate MMP inhibitors and performed microarray analysis to determine whether or not different gene pathways were affected by them. MMP-2/MMP-9 Inhibitor I [(2R)-2-[(4-Biphenylylsulfonyl)amino]-3-phenylpropionic (structure shown in Fig 1) or ilomastat (Fig 1) were used to treat mouse ears exposed to sulfur mustard using the mouse ear vesicant model (MEVM). Punch biopsies collected at various timepoints were used to perform microarray analysis. The Affymetrix microchip containing 48K mouse genes and ESTs. Principal component analysis coupled with ANOVA analysis/Welsh T test and filtered with p values less than 0.05 was used to create a gene profile list. KEGG library pathways analysis for each time period was performed and the data assigned Fisher P values. Analysis of the data revealed differences in gene pathways that were affected by the two compounds.

Results

Principal component analysis confirmed that the data separated well and was statistically significant (Fig 3). Venn Diagrams of significant gene annotations or Kegg Pathways are presented in Fig 4. Kegg Pathway Analysis resulted in different pathway profiles as shown in Fig 5. The results for MMP-2/MMP-9 Inhibitor I treatment had the greatest impact on metabolism and synthesis pathways at 24h post-exposure; primary wound repair pathways (eg junction-forming and adhesion pathways) at 72h post-exposure, and secondary repair pathways (eg proliferation pathways) at 168h post-exposure (Tables 3-5). When the specific MMP genes were examined (Fig 6), the data indicated that several of them were directly affected by the MMP inhibitors. For 24h post-exposure, many of the wound repair signaling pathways were affected, suggesting wound repair acceleration compared to the other MMP inhibitor tested. 168h post-exposure, several additional wound repair pathways were activated and several timepoints had the CAMP pathway (Fig 4, bottom). Two significant novel pathways appeared, 1) the oxidative phosphorylation pathway at 72h post-treatment with inhibitor I (Fig 7); and 2) the apoptosis pathway at 168h post-treatment with Ilomastat (Fig 8). When all the individual, specific MMP genes were expressed (Fig 6), the data indicated that several of them were directly affected by the MMP inhibitors. MMP-2/MMP-9 Inhibitor I downregulated MMP-1, MMP-10, MMP-12, MMP-9, and MMP-15 at 12.1- 2.0 x fold in at least one of the timepoints and MMP-15, MMP-19, and MMP-24 2.0 x fold or more in the 24h timepoint. Ilomastat downregulated MMP-1, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-19, MMP-23, and MMP-24 12.1 x fold or more in at least one of the timepoints. Ilomastat also decreased MMP-25 about 9.0 x fold at 24h post-exposure and MMP-24, 24h: 8.8 x fold, and MMP-19: 3.8 x fold.

Fig 4. Venn Diagrams showing the significant gene analysis (Inhibitor 1; upper left: ilomastat; upper right) or the KEGG pathways (Inhibitor 1; lower left: ilomastat; lower right) for the various time-points.

Fig 5. KEGG pathway analysis for either Inhibitor I (left panels) or Ilomastat (right panels) for 24h (upper panels); 72h (middle panels); and 168h (lower panels).

Fig 6. Table showing the fold increases or decreases for the matrix metalloproteinases that changed after application of either inhibitor I or Ilomastat for the various timepoints.

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